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(54) **Title:** PREPARATION METHOD OF VALIENAMINE FROM ACARBOSE AND/OR ACARBOSE DERIVATIVES USING TRIFLUOROACETIC ACID

(57) **Abstract:** The present invention relates to a preparation method of valienamine from acarbose and/or acarbose derivatives using organic acid TFA(trifluoroacetic acid). By using the method of the present invention, valienamine, the core precursor of voglibose which is a strong retardant of α-glucosidase and which is used for the cure of diabetes, can be produced in large quantities by using selective hydrolysis from acarbose and/or acarbose derivatives using TFA.

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PREPARATION METHOD OF VALIENAMINE FROM ACARBOSE AND/OR ACARBOSE DERIVATIVES USING TRIFLUOROACETIC ACID

Technical Field

The present invention relates to a method of producing valienamine and, more particularly, to a method of producing valienamine at a substantially high conversion rate by mass producing valienamine by way of selective hydrolysis by using TFA from acarbose and/or acarbose derivatives, and then removing therefrom its by-products, i.e., monosaccharide, disaccharide, and trisaccharide.

Background Art

The conventional art relating to commercial production of valienamine can be divided into two types. The first type relates to a method of direction production of valienamine by using microorganism fermentation, and the second type relates to a method of production of validamycin, a derivative of valienamine, by degradation by using other microorganisms.

Validamycin derivatives basically include a valienamine moiety, which selectively binds with validamine or valiolamine. Moreover, a validamycin derivative is a pseudotrisaccharide compound, which is glucose bonded in chain.

The validamycin compound is an antibiotic used for germicide for rice-cultivated land in East Asia, which is produced among other methods by culturing *Streptomyces hygroscopicus*, a soil microorganism. Here, the validamycin compound contains a small amount of intermediate valienamine, which is then separated out via a column.

As for another method for producing valienamine, there is a method of separating validamycin by using a microorganism, *F. saccharophilum*, etc. The method involves using validamycin as a substrate or medium and adding it to the liquid mixed with microorganisms; culturing them for a certain period of time and then inducing separation

of validamycin by microorganisms; and then obtaining validamycin by separation via a column. Yet, the two methods have disadvantages in that they take too much time for microorganism fermentation with not much higher yield.

Another compound which has a valienamine moiety is acarbose. Acarbose is obtained from secondary metabolic products of *Actinoplanes sp.*, which is one type of soil microorganisms. It is currently being used as a treatment for diabetes since it has inhibition effects on α -amylase. However, as of yet, there is no disclosure of the process of commercial or mass production of valienamine by using acarbose as a raw material.

As for methods of producing valienamine, reported in academia, there is a chemical method of producing valienamine by using N-bromosuccinimide (NBS) with validamycin as raw material. However, as this method uses dimethyl sulfoxide (DMSO) as solvent, it suffers from difficulties during purification and separation processes of byproducts, in addition to its low yield. Moreover, there is an ongoing research into the production method of valienamine using organic and inorganic acid, such as sulfuric acid, hydrochloric acid, and acetic acid. Yet, the method is not practical since it is limited to the extent of hydrolysis of only one terminal saccharide. Moreover, there was an attempt to produce valienamine by way of organic synthesis, but it currently is in a standstill due to the inefficiency of purification and organic synthesis processes.

There is a production method of valienamine by pre-synthesis by enzyme, which is being actively pursued in recent years. This is a method of producing valienamine by using an inexpensive substrate by finding valienamine synthesis and related enzymes expressed by a strain. However, there are many difficulties, such as determination of the degree of activity and the expression according to a gene probe. So, the current production is rather difficult.

As stated above, the production of valienamine *in vitro* by purification enzymes or chemicals has not yet been commercialized, and so up to now, valienamine has been mainly synthesized and produced by hydrolyzing validoxylamine and validamycin by

using the strains, *Pseudomonas denitrificans* and *Flavobacterium saccharophilum*. Japanese Patent No. 57,054,593 discloses a reaction of converting validoxylamine and validamycin by using microorganisms. This is a method of synthesizing and producing valienamine by using *Flavobacterium saccharophilum* by reacting of 1-5wt% mixture of validoxylamine and validamycin for 24~200 hours at reaction conditions of 20-45℃ and pH 5-8.

Summary of the Invention

The present invention purports to provide a method of producing valienamine at a substantially high conversion rate by first mass producing valienamine through selective hydrolysis from acarbose and/or acarbose derivatives by using TFA, and then removing byproducts, i.e., monosaccharide, disaccharide, and trisaccharide.

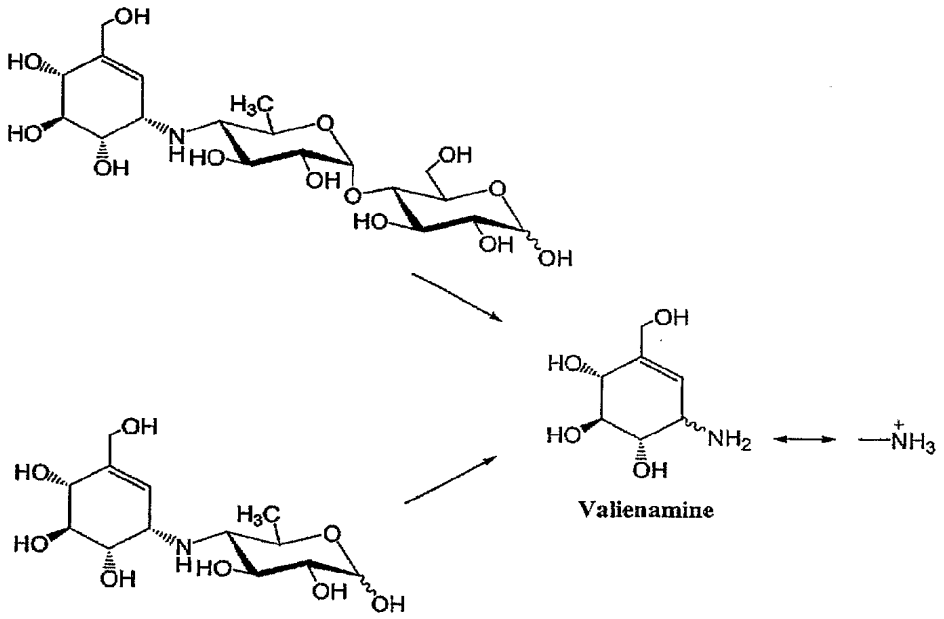
To achieve said objectives, the present invention involves a method of producing valienamine from acarbose and/or acarbose derivatives by using trifluoroacetic acid (TFA). In particular, the present invention provides a method of producing valienamine by using a reaction substrate of final concentration of 0.2-10% acarbose and/or acarbose derivatives, and a reaction solvent of 10-60% TFA solution.

If the final concentration of acarbose and/or its derivatives is less than 0.2%, or that of TFA exceeds 60%, the production cost per unit increases. On the other hand, if the final concentration of acarbose and/or its derivatives exceeds 10%, or that of TFA is less than 10%, the yield therein decreases.

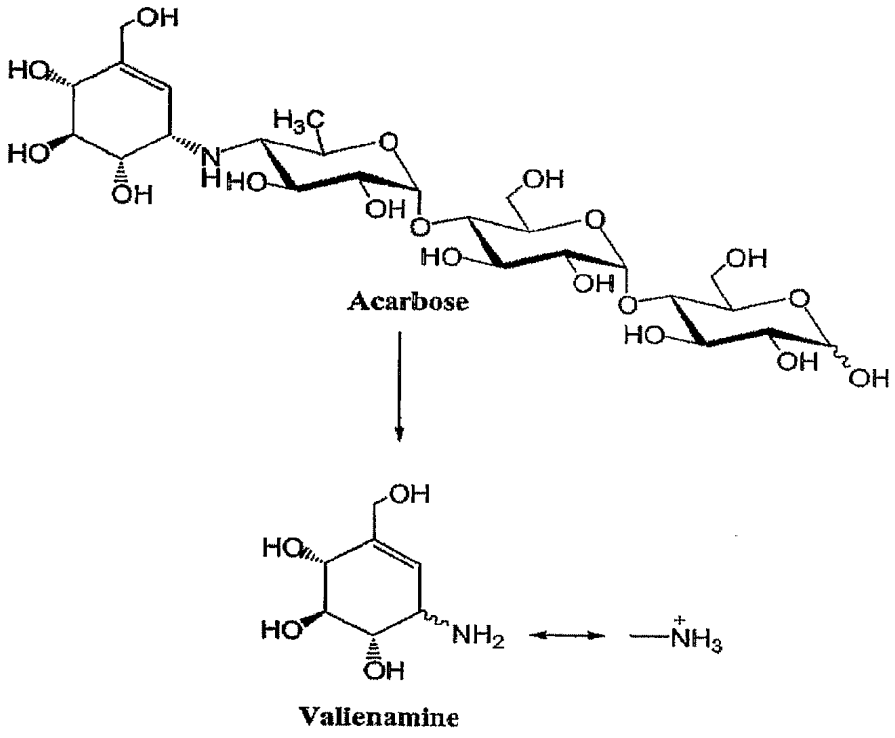
Moreover, the present invention provides a method of producing valienamine from acarbose and/or acarbose derivatives by using TFA, which is characterized by reacting it for 1~24 hours at 80~120℃, or by using a high-temperature and high-pressure autoclave, which can reduce reaction time to one hour and increase its yield up to 96%.

Accordingly, the present invention can yield valienamine with an amine group of NH_2 or NH^+_3 at its carbon chain.

Formula 1



Formula 2



Moreover, according to the present invention, an acarbose derivative is a compound having one, two, four, five or more saccharides bonded to a carbon chain, but generally refers to a derivative of one or two saccharides.

Valienamine is known to have maltase and sucrase inhibition effects and to have antibiotic activity as against *Bacillus* species. Moreover, its intramolecular atom alignment is similar to that of alpha-D-glucose. The inhibition activity of alpha-glucosidase of valienamine is believed to be caused by structural similarity of valienamine to D-glucosyl cation. The D-glucosyl cation with an enzyme as a catalyst forms a half-chair conformation in a transition state, which is produced during hydrolysis of pyranoside.

As for compounds with a valienamine moiety, there are acarbose, its derivatives, validoxylamine, validamycin, etc. Among these, acarbose is being widely used as an inhibitor for Type II diabetics. Acarbose and acarbose derivatives have different structures from the other two compounds (validoxylamine and validamycin), and their productions methods are substantially different as well.

Compounds with a valienamine moiety, i.e., acarbose, its derivatives, validoxylamine, and validamycin, are all potentially raw materials for valienamine. All of them are produced by fermentation by different bacteria strains, respectively. Among these, acarbose is being distributed as a diabetic treatment all over the world by a German pharmaceutical company, Bayer, Inc., along with Chinese and Japanese pharmaceutical companies. Acarbose is more expensive than validamycin but is easier to obtain in a pure raw material form. Accordingly, acarbose has the advantage of easy separation process, which makes it an appropriate raw material for valienamine. When using acarbose as a raw material, there is a problem of difficult purification by way of pigments from cleaved saccharide, but this can be easily resolved by using acarbose derivatives.

Brief Description of Drawings

Fig. 1 is a hydrogen NMR spectrum of valienamine produced from acarbose by using TFA.

Fig. 2 is a carbon NMR spectrum of valienamine produced from acarbose by using TFA.

Fig. 3 is a hydrogen NMR spectrum of valienamine produced from an acarbose derivative by using TFA.

Fig. 4 is a carbon NMR spectrum of valienamine produced from an acarbose derivative by using TFA.

Fig. 5 is a graph of Bio-LC(HPLC) data of valienamine produced from an acarbose derivative by using TFA.

Detailed Description of the Preferred Embodiment

Example 1: Method of producing valienamine using TFA

10g of pure acarbose were place into 10% TFA solution at 5% final concentration. At reaction temperature of 100℃, it was reacted for 12 hours or more, followed by removal of TFA and water. Then, by using ion-exchange resins for purification, 2.02g of valienamine were obtained.

Example 2: Method of producing valienamine using TFA

1g of a pure acarbose derivative (monosaccharide and trisaccharide) were place into 10% TFA solution at 5% final concentration. At reaction temperature of 100℃, it was reacted for 12 hours or more, followed by removal of TFA and water. Then, by using ion-exchange resins for purification, 0.45g and 0.31g of valienamine were obtained, respectively.

Example 3: Method of producing valienamine using TFA by using an autoclave

10g of pure acarbose were place into 10% TFA solution at 5% final concentration.

While putting pressure using an autoclave, at reaction temperature of 121℃, it was reacted for 30 minutes to 1 hour, followed by removal of TFA and water. Then, by using ion-exchange resins for purification, 2.1g of valienamine were obtained.

Example 4: Method of producing valienamine using TFA by using an autoclave

1g of a pure acarbose derivative (monosaccharide and trisaccharide) were place into 10% TFA solution at 5% final concentration. While putting pressure using an autoclave, at reaction temperature of 121℃, it was reacted for 30 minutes to 1 hour, followed by removal of TFA and water. Then, by using ion-exchange resins for purification, 0.46g and 0.30g of valienamine were obtained, respectively.

The hydrogen and carbon NMR spectrums with respect to the resultant products obtained as a result of the reactions of Examples 1 and 2 are as follows: ^{1}H-NMR(D_2O) δ : 3.42(1H, br s, H-1), 3.54(2H, Abq, J=13.6Hz, H-7), 3.94(1H, d, J=6.79Hz), 3.97(1H), 4.05(1H), 5.64(1H, d, J=4.6) ^{13}C-NMR(D_2O) δ : 48.9(C-1), 61.2(C-7), 69.7(C-2), 71.7(C-4), 72.0(C-3), 123.4(C-6), 139.9(C-5).

Industrial Applicability

By using the method of the present invention, valienamine can be produced from acarbose with yield rate of 50~95%, and/or acarbose derivatives with yield rate of 70~95%. Since hydrolysis is occurred on the α-binding adjacent to the amine moiety of valienamine, only monosaccharide, disaccharide or trisaccharide are produced as byproducts. Due to this advantage, the refining process becomes simple making it possible to produce valienamine with high purity while reducing the pigments.

In addition, by using the method of the present invention, the voglibose, which is widely sold as a remedial agent of diabetes over the world including Korea, Japan and China, can be more easily produced cutting down the production cost. Also the invention can contribute to the development of valienamine derivatives which have better pharmaceutical activity, or which can be used on other types of disease.

CLAIMS

What is claimed is:

1. A method of producing valienamine from acarbose and/or an acarbose derivative by using trifluoracetic acid (TFA).

2. The method of producing valienamine according to Claim 1 from acarbose and/or an acarbose derivative by using trifluoroacetic acid (TFA), which comprises using a reaction substrate of final concentration of 0.2~10% of acarbose and/or an acarbose derivative.

3. The method of producing valienamine according to Claim 1 from acarbose and/or an acarbose derivative by using trifluoroacetic acid (TFA), which comprises using 10~60% of TFA.

4. The method of producing valienamine according to Claim 1 from acarbose and/or an acarbose derivative by using trifluoroacetic acid (TFA), which comprises carrying out reactions for 1~24 hours at 80-120℃.

5. The method of producing valienamine according to Claim 1 from acarbose and/or an acarbose derivative by using trifluoroacetic acid (TFA), which comprises using an autoclave at high temperature and pressure for reducing reaction time and increasing the yield and.

Fig. 1

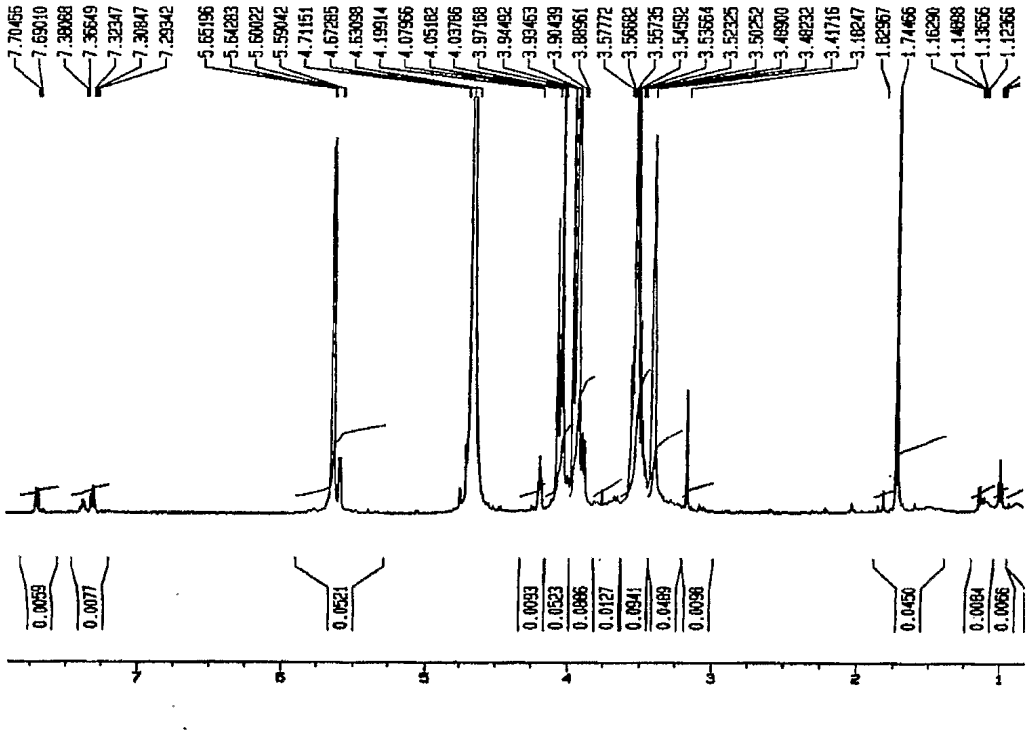

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Fig. 2

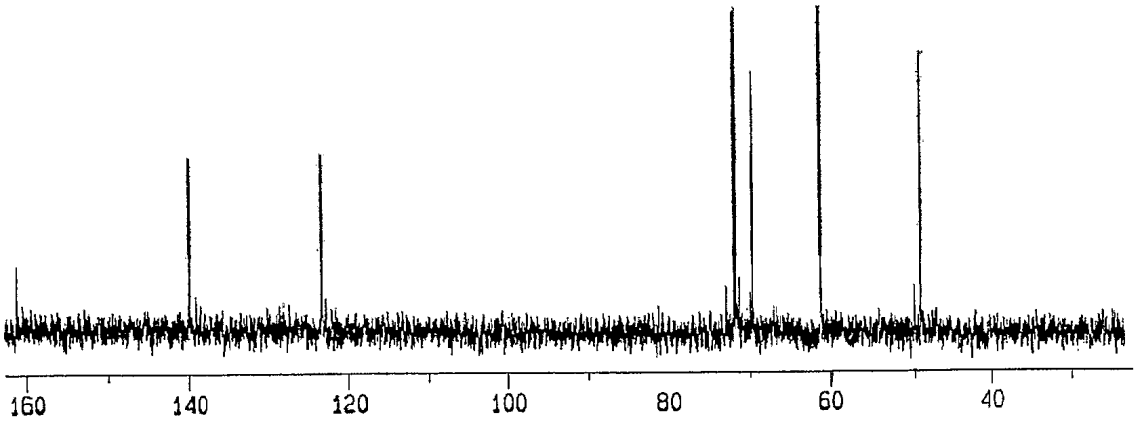
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Fig. 3

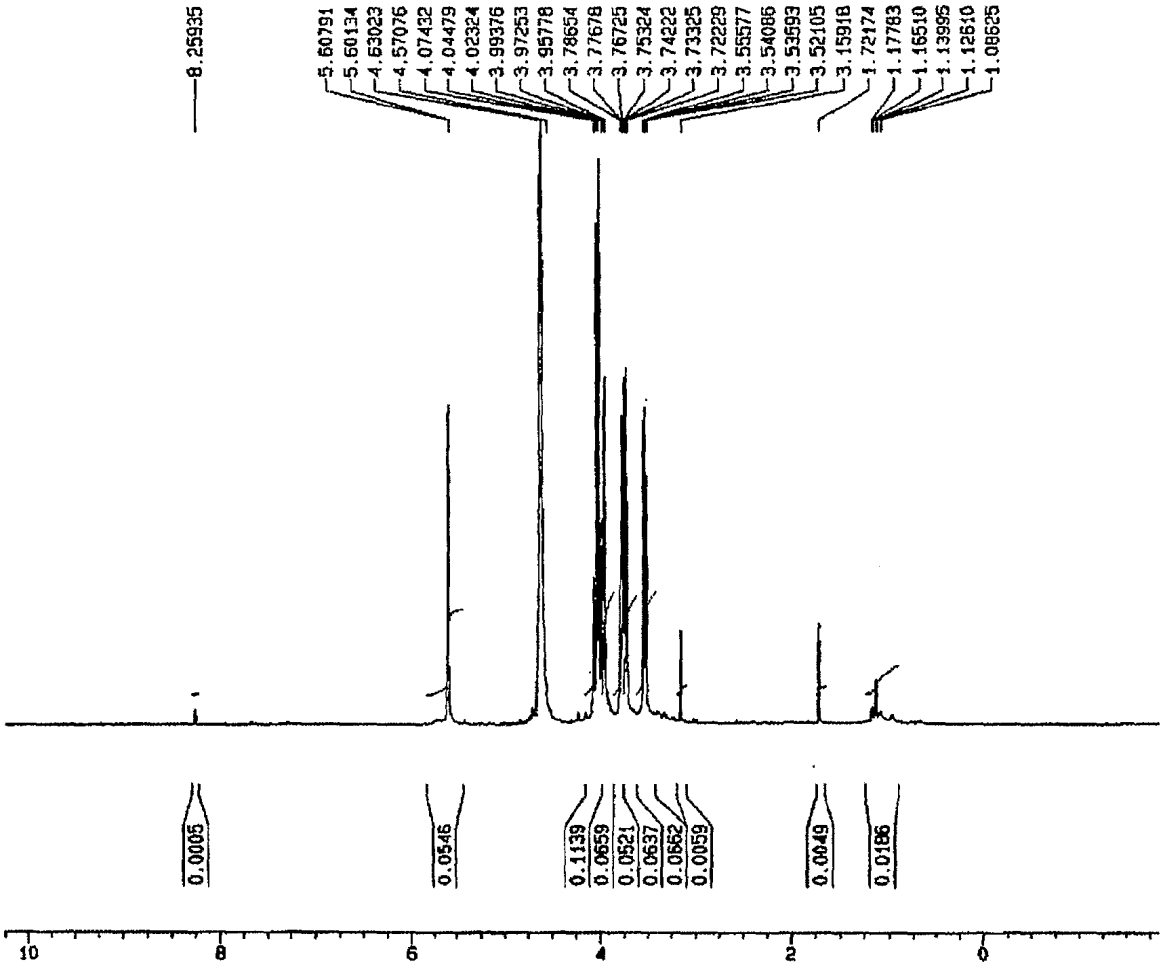
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Fig. 4

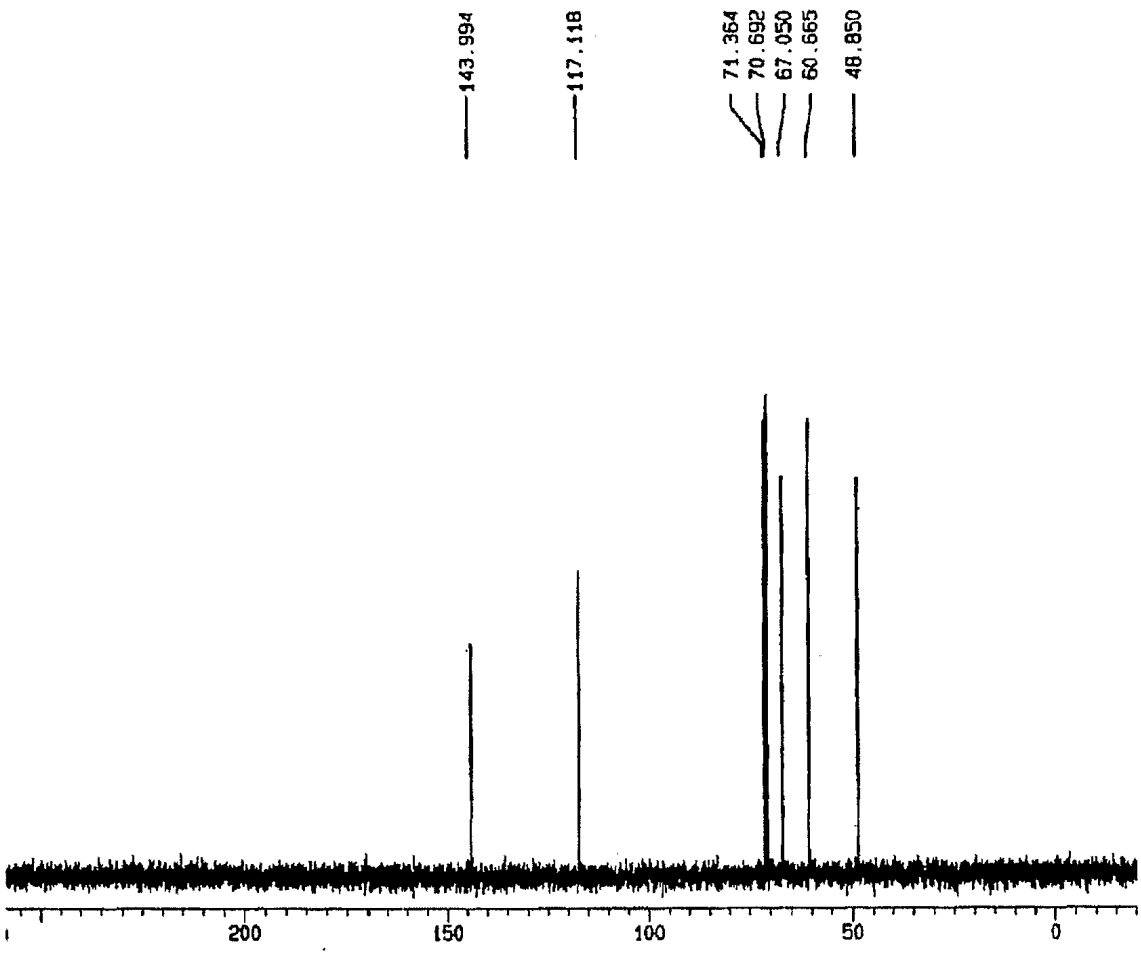
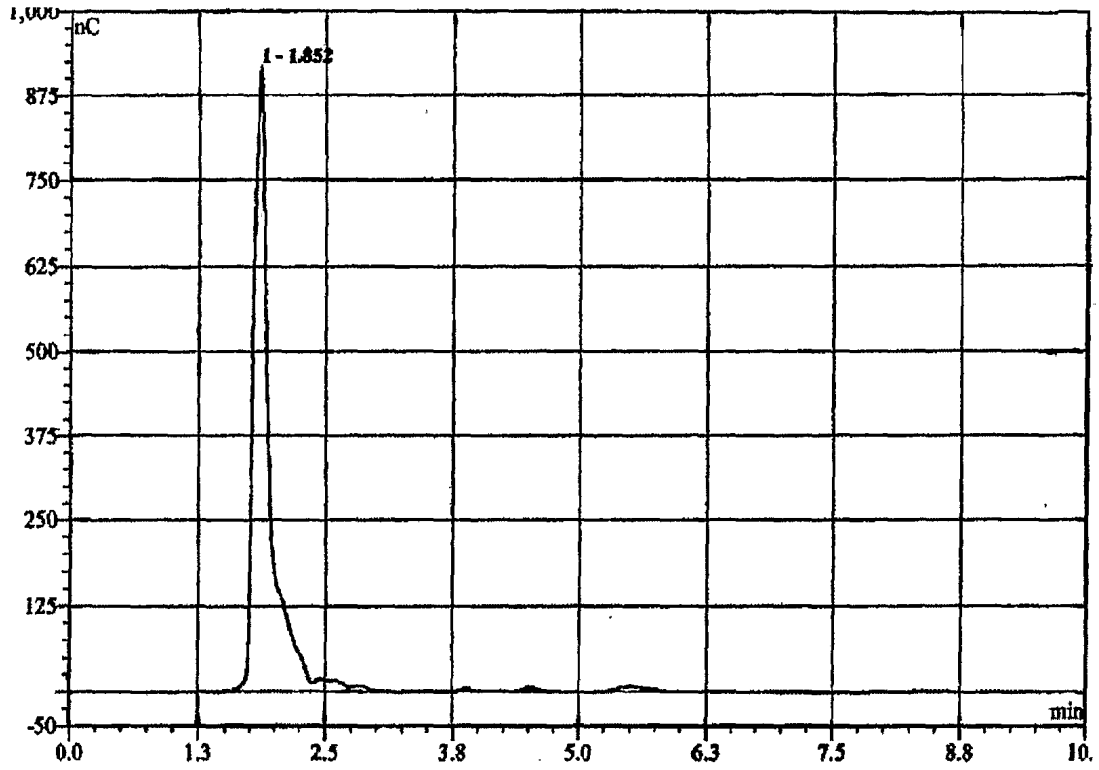


Fig. 5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10.

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/KR02/02198

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07C 209/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7 ; C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
KR ; IPC as above

Electronic data base consulted during the internationl search (name of data base and, where practicable, search terms used)
STN (Registry, Caplus; keyword & role search)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Tatsuta, Kuniaki et al, "Novel synthesis of natural pseudo-aminosugars, (+)-valienamine and (+)-validamine", J. of Antibiotics, 2000 53(4), page 430-435 see Figure 3 at page 431	1-10
A	Ogawa, Seichiro et al, "Synthetic studies on antibiotic validdmycin A and related compound: synthesis of branched-chain and unsaturated branched-chain aminocyclitiols", Kenkyu Hokoku-Asahi Garasu Kogyo Gijutsu Shoreikai, 1983 (43), page 127-133 see Figure 4 at page 128	1-10
A	Yoshikawa, Msayuki et al, "Syntheses of validamine, epi-validamine, and valienamine, three optically active pseudo-amino-sugars, from D-glucose", Chemical and Pharmaceutical Bulletin, 1988 36(10), page 4236-4239 see whole document ----------End of documents----------	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 JANUARY 2003 (09.01.2003)	10 JANUARY 2003 (10.01.2003)

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